## REMARKS

By the present Amendment, Applicants have canceled pending Claims 1-45 in favor of newly added Claims 46-59. Claims 1-45 are canceled without prejudice or disclaimer as to the subject matter contained therein. Applicants reserve the right to file continuation and/or divisional applications on all or a portion of the subject matter which was canceled by this Amendment.

Applicants present the instant Amendment in conjunction with a Request By Applicants For Interference Pursuant to 37 CFR § 1.607, wherein Applicants respectfully request that an interference be declared between the above referenced application and U.S. Patent No. 6,174,673. The information required by 37 CFR § 1.607(a) is set forth under headings which correspond to the subsections of § 1.607 to facilitate consideration by the Examiner.

Support for the amendments to the specification can be found in the specification as originally filed and as amended by incorporated disclosure of the cited publication by Mairin B. Brennan, "Enzyme Discovery Heats Up", *Chem. & Eng. News* 74: 31-33, 1996. Brennan is cited at page 12 of the specification and is incorporated by reference at page 95, lines 3-5 and 25 ("The following references, . . . .are specifically incorporated herein by reference."). Specific support for each element of the claims can be found in **Appendix A**, attached hereto. A copy of the Brennan reference is submitted herewith for the Examiner's convenience. The

material incorporated by reference can be found in the following sections of the Brennan reference:

-Page 31, Column 2, 1st full paragraph

-Page 31, paragraph bridging Columns 2 and 3

-Page 31, Column 3, 4th full paragraph

-Page 31, Column 3, last paragraph

-Page 32, Column 1, "Focus" and "Products" sections of Box

-Page 32, Column 1, 1st paragraph under Box

-Page 32, Column 1, second to last line

-Page 32, Column 3, flow chart

-Page 33, Column 3, lines 9-10

## REQUEST FOR INTERFERENCE

### I. IDENTIFICATION OF THE PATENT WHICH INCLUDES SUBJECT MATTER WHICH INTERFERES WITH THE APPLICATION

The patent which claims subject matter which interferes with subject matter claimed in the present application ("the '672 application") is U.S. Patent No. 6,174,673 ("the '673 patent") issued on January 16, 2001 to Jay M. Short *et al.* for "High throughput screening for novel enzymes". The '673 patent was issued on application Serial No. 09/098,206, filed

January 16, 1998, which purports on its face to be a continuation-in-part of 08/876,276, filed on June 16, 1997. Diversa Corporation is the assignee named on the face of the patent.

## II. PRESENTATION OF A PROPOSED COUNT

Attached **Appendix B** sets forth the proposed Count. The proposed Count is an alternative Count prepared after consideration of the subject matter claimed by the respective parties. As required by 37 CFR § 1.601(f), the proposed Count "defines the interfering subject matter between . . . one or more applications and one or more patents."

The Count is proposed in alternative form because of the slightly different language utilized by the respective parties to describe the same invention. The interfering subject matter between Iverson and Short relates to methods for identifying polypeptides using high throughput screening of genomic DNA from mixed populations of organisms. The proposed Count comprises claim 1 of the '673 patent and claim 46 of the instant application set forth in the alternative.

## III. IDENTIFICATION OF CLAIM OF THE '673 PATENT WHICH CORRESPONDS TO THE PROPOSED COUNT

Claims 1-23 of the '673 patent are believed to correspond to the proposed Count. Claims 1 and 22 are independent claims. Claim 1 of the '673 patent is directed to a method of identifying the bioactivities or biomolecules using high throughput screening of genomic DNA.

Claim 22, although an independent claim, is a species of claim 1. Claim 22, like claim 1 of the '673 patent, is directed to a method for identifying bioactivities or biomolecules using high throughput screening of genomic DNA, but is limited to a method wherein the genomic DNA is prokaryotic DNA, and wherein the method is performed in a culture-independent manner. The method of claim 22 is thus a species of the method of claim 21. Claim 23 is dependent on claim 22 and is part of the Count for the same reasons as set forth for claim 22.

In order to assist the Examiner, attached **Appendix C** sets forth a side-by-side comparison of claims 1-23 of the '673 patent with the proposed Count.

## IV. CLAIMS OF THE '672 APPLICATION WHICH CORRESPOND TO THE PROPOSED COUNT

Previously pending claims 1-45 have been canceled without prejudice or disclaimer as to the subject matter contained therein. New claims 46-59 have been added. New claims 46-59 are believed to correspond to the proposed Count. To assist the Examiner in this regard, Applicants attach **Appendices A** and **D**. **Appendix A** is a chart providing an element-by-element recitation of the newly added claims of the '672 application, and an indication of the passages in the originally filed application[1] where, at the very least, the claims find support.

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[1] The present '672 application was filed February 12, 2001 and is a continuation/divisional of the '063 application, filed on May 1, 1997. The '673 Patent was filed January 16, 1998 and purports to be a continuation-in-part of Serial No. 08/876,276 filed

(continued...)

**Appendix D** is a chart providing a side-by-side comparison of new claims 46-59 of the '672 application with the proposed Count, which explains the rationale for including these claims in the interference based on the proposed Count.

## V. 35 U.S.C. § 135(b) IS SATISFIED

At least one claim is being submitted in the above referenced application which is the same as, or for the same as, or substantially for the same subject matter as a claim of the '673 patent, and such claim is being made prior to one year from the date on which the '673 patent was granted (*i.e.*, January 16, 2001). Thus, § 135(b) is satisfied.

## VI. CONCLUSION

Applicants respectfully request that an interference be declared employing the proposed Count set forth on attached Appendix A, with claims 1-23 of the '673 patent and claims 46-59 of the present application designated as corresponding the Count. Such action is respectfully requested.

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(...continued)
June 16, 1997. Accordingly, even if Short were accorded benefit of its earliest filed application, Iverson should be designated Senior Party in the interference.

Should the Examiner feel that there are any issues outstanding after consideration of this response, the Examiner is invited to contact Applicants' undersigned representative to expedite prosecution.

Respectfully submitted,

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Date: August 2, 2001

Application No. 09/782,672
Attorney's Docket No. 032705-002



**Attachment to Preliminary Amendment dated August 1, 2001**

Marked-up Copy

Page 12, Paragraph Beginning at Line 2

Efforts to engineer improved enzymes rely upon molecular biology techniques and involve two basic approaches. The first involves cloning and expression of enzyme libraries from organisms that cannot be cultivated and typically are isolated from extreme environments. The exotic microorganisms that inhabit scalding hot springs, freezing Arctic waters, sulfur-rich geothermal springs, highly saline waters, or extremely acid or alkaline habitats are called extremophiles, and their enzymes are dubbed extremozymes. Precisely because extremophiles thrive in such outrageous environments, they are tricky to grow in laboratory cultures, the conventional first step toward isolating products an organism secretes. One advantage of using prokaryotes, which include most extremophiles, is that their genes are linked together. So far, most of the extremozymes RBI has discovered have come from hyperthermophiles - microorganisms that thrive at temperatures that often exceed 100°C. This approach, pioneered by Recombinant Biocatalysis (Brennan, 1996), relies on the construction expression libraries by extracting DNA from samples and gene amplification by PCR™. Bypassing the culture hurdle, Recombinant BioCatalysis Inc. (RBI) in Sharon Hill, Pa., has gone straight to the target - shotgun cloning DNA from a mix of organisms to fast-forward discovery of the enzymes they produce. RBI's focus is on the discovery, development and commercialization

of enzymes from microorganisms that live in biodiverse environments to provide biocatalysts for the pharmaceutical and chemical process industries, including glycosidases, lipases, aminotransferases, phosphatases, cellulases, esterases, catalysts for chiral resolutions and for peptide synthesis. The expression libraries are then screened by brute force approaches that rely heavily on [robotics] an accelerated robotic screening system that handles several different enzyme assays simultaneously and operates 24 hours a day.

Fast track to enzymes from extremophiles

Obtain biomass samples from extreme environments

↓

Extract and purify DNA

↓

Clone DNA segments to generate genomic gene expression libraries

↓

Pool the expression products of several clones and screen for enzyme activity

↓

Establish DNA sequence of gene encoding an enzyme

↓

Subclone DNA sequence for large-scale expression of the enzyme

↓

Optimize enzyme by random DNA mutagenesis.

This technology promises to begin to tap the unexplored diversity of function in the natural world. However, it is intrinsically limited to catalytic activities that serve a biological function. Also, it is limited by the ability to employ genes from unknown sources to direct the synthesis of functional properties in commonly used host organisms such as *E. coli* or *Saccharomyces cerevisiae*.

Page 95, Paragraph Beginning at Line 25

Brennan, Chemical and Eng. News, [74:31033] 74:31-33, 1996

## APPENDIX A

### New Claim Chart

| New Claims 46-59 | Support in the Specification[1] |
|---|---|
| **46.** A method for identifying polypeptides having a desired activity | p. 1, l. 14 ["identify specific polypeptides having desirable characteristics"]; p. 3, l. 7 ["antibodies with particular binding functions, as well as other activities"]; p. 4, l. 18 ["biocatalyst"]; p. 5, l. 16 ["enzyme activity"]; p. 11, ll. 21-22 ["biologically active polypeptides, in terms of both function and specificity"]; p. 15, ll. 17-19 ["to provide a general approach for the efficient screening of very large libraries of virtually any polypeptide for desirable activity"]; p. 23, l. 19 ["Cells exhibiting the desired activity will be isolated"]; and Claim 4 as originally filed. |

[1] This support is exemplary only; support is not intended to be limited to that disclosure specifically referenced.

| New Claims 46-59 | Support in the Specification[1] |
|---|---|
| using high throughput screening | p. 7, l. 18 - p. 8, l. 4 ["rapid and efficient screening"]; p. 12, ll. 8-9 ["screened by brute force approaches that rely heavily on robotics"] p. 15, ll. 17-19 ["to provide a general approach for the efficient screening of very large libraries of virtually any polypeptide for desirable activity"]; p. 32, l. 10 ["high throughput"]; p. 54, ll. 24-25 ["A particularly preferred method for identification and isolation is cell sorting or flow cytometry. One aspect of this method is fluorescence activated cell sorting"]; p. 62, ll. 1-19; p. 64, ll. 27-28 ["rapid measurement of large numbers of individual microcolonies by flow cytometry"]; see also, material incorporated by reference at p. 12, l. 9 ["an accelerated robotic screening system that handles several different enzyme assays simultaneously and operates 24 hours a day"]; and Claims 22-24, 35-37 as originally filed. |
| of genomic DNA comprising: | Support can be found throughout the specification for DNA; see also material incorporated by reference at p. 12, l. 9 ["genomic gene expression libraries"] |
| **a)** providing an expression library containing a plurality of clones, | p. 54, ll. 3-4 ["a library of cell surface displayed proteins is prepared"]; p. 61, ll. 22-23 ["a cell population where each cell displays a different polypeptide"]; see also; and Claim 11 as originally filed. |

| New Claims 46-59 | Support in the Specification[1] |
|---|---|
| wherein the DNA for generating the library is obtained from a mixed population of organisms | See material incorporated by reference at p. 12, l. 8 ["mix of organisms"] |
| **b)** enclosing a fluorescent substrate and at least one clone from the library | p. 67, ll. 12-22. ["the enzymatic reaction with substrate is carried out in AGM's with enclosed bacteria from the surface-expressed enzyme library"]; and p. 65, ll. 21-22 ["a substrate can synthesized that has a fluorophore"].<br><br>*AGMs are "agarose gel microdroplets". |
| in a gel microdroplet | p. 63, l. 16-p. 65, l. 6 ["gel microdroplet"]; p. 67, ll. 12-22. ["the enzymatic reaction with substrate is carried out in AGM's with enclosed bacteria from the surface-expressed enzyme library."]; p. 63, l. 15 to p. 64, l. 15; and p. 67, ll. 12-22.<br><br>*AGMs are agarose gel microdroplets. |
| wherein the substrate is fluorescent in the presence of the polypeptide having the desired activity | p. 65, l. 8 to p. 66, l. 13. ["Identification of mutant enzymes with desirable properties such as novel substrate selectivity or remarkable catalytic activity can be achieved using substrates that change an assayable property. *i.e.* fluorescence intensity, ratio of multiple fluorophore emissions, antibody detectable structural changes *etc.*, upon catalytic action of the enzyme."] |

| New Claims 46-59 | Support in the Specification[1] |
|---|---|
| c) screening the microdroplet with a fluorescent analyzer that detects fluorescence; and | p. 67, ll. 12-22 ["the AGM's with desired enzyme activities could be isolated by FACS or via fluorescence microscopy using a micromanipulator"]; Example 2, p. 73, ll. 27-28 ["cells having an allowable fluorescent signal"]; Example 7, especially p. 88, ll. 1-24; Example 8, pp. 91-92; and Example 9, pp. 93-94. |
| d) identifying clones detected as positive for fluorescence, wherein fluorescence is indicative of DNA that encodes the polypeptide having the desired activity. | p. 67, ll. 12-22. ["the AGM's with desired enzyme activities could be isolated by FACS or via fluorescence microscopy using a micromanipulator."]. |
| **47.** The method of claim 46, wherein the polypeptide is an enzyme selected from the group consisting of lipases, esterases, glycosidases, phosphatases, aminotransferases and cellulases. | p. 7, ll. 18-27 ["the present invention provides methods for the rapid screening of enzyme libraries"]; and *see also*, material incorporated by reference at p. 12, l. 8 ["glycosidases, lipases, aminotransferases, phosphatases, cellulases, esterases"]. |
| **48.** The method of Claim 46, wherein the polypeptide is an enzyme which catalyzes chiral resolutions. | *See* material incorporated by reference at p. 12, l. 8 ["catalysts for chiral resolution"]. |
| **49.** The method of Claim 46, wherein the polypeptide is an enzyme which catalyzes peptide synthesis. | *See* material incorporated by reference at p. 12, l. 8 ["catalysts for peptide synthesis"]. |
| **50.** The method of Claim 46, wherein the library is generated in a prokaryotic cell. | p. 16, l. 12 ["*E. coli* bacteria"]; see also, Tables on pp. 17-18 and 19-20; p. 24, ll. 14-27; p. 49, ll. 16-19; and Claims 2 and 3 as originally filed. |

| New Claims 46-59 | Support in the Specification[1] |
|---|---|
| **51.** The method of Claim 50, wherein the prokaryotic cell is gram negative. | p. 16, l. 20; p. 18, l. 7; p. 21, l. 14; p. 24, ll. 14-27; p. 49, ll. 16-19 ["Gram-negative bacteria"]; and Claims 2 and 42 as originally filed. |
| **52.** The method of Claim 49, wherein the prokaryotic cell is *E. coli*. | p. 16, l. 12 ["*E. coli* bacteria"]; *see also*, Tables on pp. 17-18 and 19-20; p. 24, ll. 14-27; p. 49, ll. 16-19; and Claim 3 as originally filed. |
| **53.** The method of Claim 46, wherein the expression library contains DNA obtained from extremophiles. | p. 3, l. 24 ["isolation of thermostable variants"]; p. 12, ll. 4-6 ["cloning and expression of enzyme libraries from organisms that cannot be cultivated and typically are isolated from extreme environments"]; and *see also*, material incorporated by reference at p. 12, l. 5 ["extremophiles"]. |
| **54.** The method of Claim 53, wherein the extremophiles are thermophiles. | p. 3, l. 24 ["isolation of thermostable variants"]; and *see also*, material incorporated by reference at p. 12, l. 5 ["hyperthermophiles"]. |
| **55.** The method of Claim 53 wherein the extremophiles are selected from the group consisting of hyperthermophiles, psychrophiles, halophiles, psychrotrophs, alkalophiles, and acidophiles. | p. 3, l. 24 ["isolation of thermostable variants"]; and *see also*, material incorporated by reference at p. 12, l. 5 ["hyperthermophiles"; "microorganisms that inhabit scalding hot springs, freezing Arctic waters, sulfur-rich geothermal springs, highly saline waters or extremely acid or alkaline habitats"]. |
| **56.** The method of Claim 46, wherein the fluorescent analyzer comprises a FACS apparatus. | p. 54, ll. 24-25 ["One aspect of this method is fluorescence activated cell sorting"]. |

| New Claims 46-59 | Support in the Specification[1] |
|---|---|
| **57.** The method of Claim 46, including the additional steps of: subjecting an enzyme encoded by the DNA identified in step d) to directed evolution comprising the steps of: | p. 7, ll. 18-20 ["the present invention provides methods for the rapid screening of enzyme libraries. The libraries represent mutagenized version of an enzyme to permit for the 'directed' evolution of the enzyme's sequence, and hence function."]. |
| **a)** subjecting the enzyme to non-directed mutagenesis; and | p. 4, l. 4-p. 5, l. 28 ["a variety of techniques including chemical mutagenesis of isolated DNA, gene amplification by error prone PCR™ and oligonucleotide mutagenesis have been employed to generate libraries of mutant genes containing a desired range of nucleotide substitutions."]; and p. 25, l. 15-p. 32, l. 24. |
| **b)** screening mutant enzymes produced in step a) for a mutant enzyme. | p. 7, ll. 18-19 ["the present invention provides methods for the rapid screening of enzyme libraries"]. |
| **58.** The method of Claim 46, wherein the DNA for generating the library is genomic DNA from a prokaryote. | p. 16, l. 12 ["*E. coli* bacteria"]; *see also*, Tables on pp. 17-18 and 19-20; p. 24, ll. 14-27; p. 49, ll. 16-19; and Claims 2 and 3 as originally filed. |
| **59.** The method of claim 58, wherein the DNA for generating the library is obtained using a culture-independent system. | *See* material incorporated by reference at p. 12, ll. 15 *et seq.* ["Bypassing the culture hurdle. . ."] |

# APPENDIX B

| SOURCE | PROPOSED COUNT |
|---|---|
| **Claim 1 of the '673 Patent** | A method for identifying bioactivities or biomolecules using high throughput screening of genomic DNA comprising:<br>a) providing an expression library containing a plurality of clones, wherein the DNA for generating the library is obtained from a mixed population of organisms;<br>b) encapsulating a bioactive fluorescent substrate and at least one clone of the library in a gel microdroplet, wherein the substrate is fluorescent in the presence of a bioactivity or biomolecule;<br>c) screening the microdroplet with a fluorescent analyzer that detects bioactive fluorescence; and<br>d) identifying clones detected as positive for bioactive fluorescence, wherein fluorescence is indicative of DNA that encodes a bioactivity or biomolecule. |
| | OR |
| **Claim 46 of the '672 application** | A method for identifying polypeptides having a desired activity using high throughput screening of genomic DNA comprising:<br>a) providing an expression library containing a plurality of clones, wherein the DNA for generating the library is obtained from a mixed population of organisms<br>b) enclosing a fluorescent substrate and at least one clone from the library in a gel microdroplet wherein the substrate is fluorescent in the presence of the polypeptide having the desired activity<br>c) screening the microdroplet with a fluorescent analyzer that detects fluorescence; and<br>d) identifying clones detected as positive for fluorescence, wherein fluorescence is indicative of DNA that encodes the polypeptide having the desired activity. |

## APPENDIX C

**Comparison of the '673 Patent Claims with Proposed Count**

| '673 Claim | Count |
|---|---|
| **1.** A method for identifying bioactivities or biomolecules using high throughput screening of genomic DNA comprising:<br>a) providing an expression library containing a plurality of clones, wherein the DNA for generating the library is obtained from a mixed population of organisms;<br>b) encapsulating a bioactive fluorescent substrate and at least one clone of the library in a gel microdroplet, wherein the substrate is fluorescent in the presence of a bioactivity or biomolecule;<br>c) screening the microdroplet with a fluorescent analyzer that detects bioactive fluorescence; and<br>d) identifying clones detected as positive for bioactive fluorescence, wherein fluorescence is indicative of DNA that encodes a bioactivity or biomolecule. | Claim 1 is identical to the first alternative of the Count. |

| '673 Claim | Count |
|---|---|
| **2.** The method of claim 1, wherein the bioactivity is an enzyme is selected from the group consisting of lipases, esterases, proteases, glycosidases, glycosyl transferases, phosphatases, kinases, mono- and dioxygenases, haloperoxidases, lignin peroxidases, diarylpropane peroxidases, epozide hydrolases, nitrile hydratases, nitrilases, transaminases, amidases, and acylases. | Claim 2 is dependent on Claim 1. Claim 2 further provides specific enzymes to be identified by the method. Claim 2 is obvious over the proposed Count given that at least Brennan suggests several of these enzymes as good targets for enzyme discovery. *Chem. & Eng. News* 74:31-33, 31 (1996). |
| **3.** The method of claim 1, wherein the library is generated in a prokaryotic cell. | Claim 3 is dependent on Claim 1. Claim 3 further provides for the expression library being generated in a prokaryotic cell. Claim 3 is obvious over the proposed Count given that expression libraries were commonly prepared in prokaryotic cells, such as *E. coli*. *See, e.g.*, Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL 17.11-17.36 (2nd ed. Cold Spring Harbor, NY, 1989). |
| **4.** The method of claim 3, wherein the prokaryotic cell is gram negative. | Claim 4 is dependent on Claim 3. Claim 4 further provides that the expression library be prepared in a gram negative cell. Claim 4 is obvious over the proposed Count given that expression libraries were commonly prepared in *E. coli*, a gram negative prokaryote. *Id. See also*, U.S. Patent No. 5,149,639 at col. 8 *et seq.*: "Example 5 Construction of a Genomic Library of *Streptomyces antibioticus* ATCC 11891 in pNJ1 and introduction into *E. coli*." |

| '673 Claim | Count |
|---|---|
| **5.** The method of claim 4, wherein the prokaryotic cell is *E. coli*. | Claim 5 is dependent on claim 4. Claim 5 further provides for the expression library being prepared in *E. coli*. Claim 5 is obvious in view of the proposed Count, because expression libraries were commonly prepared in *E. coli*. *Id.* and *see also* Example 5 of U.S. Patent No. 5,149,639. |
| **6.** The method of claim 5, wherein prior to step b), the *E. coli* is transferred to a Streptomyces sp. | Claim 6 is dependent on claim 5. It is assumed that Claim 6 is meant to provide that the DNA expression library prepared in *E. coli* can be transferred to a Streptomyces sp. Given this assumption, Claim 6 is obvious in view of the proposed Count, because the transfer of libraries between species was known to the skilled artisan. For example, *see* Bormann *et al.*, *J. Bacteriol.* 178: 1216-8 (1996), which teaches the expression of a genomic library obtained from *S. tendae* and expressed in *S. lividans* TK23 by the use of cosmids. See also, Example 6 of U.S. Patent No. 5,149,639 which describes the transfer of the *Streptomyces antibioticus* library from *E. coli* to *Streptomyces erythreus* (col. 9, ll. 1*et seq.*). |

| '673 Claim | Count |
|---|---|
| **7.** The method of claim 6, wherein the Streptomyces sp. is *Streptomyces venezuelae*. | Claim 7 is dependent on claim 6. Thus the assumption for claim 6 applies for claim 7. Therefore, claim 7 further provides that the Streptomyces species is *Streptomyces venezuelae*. Claim 7 is obvious in view of the proposed Count, because the skilled artisan would have known that the DNA could be transferred into different *Streptomyces* species, including *S. venezuelae*. *See*, *e.g.*, U.S. Patent No. 5,149,639 at col. 14, ll. 39-47. |
| **8.** The method of claim 1, wherein the expression library contains DNA obtained from extremophiles. | Claim 8 is dependent on claim 1. Claim 8 further provides that the expression library DNA is obtained from extremophiles. Claim 8 is obvious over the proposed Count in view of at least Brennan (1996) at 31, which suggests preparing libraries from extremophiles to identify proteins. |
| **9.** The method of claim 8, wherein the extremophiles are thermophiles. | Claim 9 is dependent on claim 8. Claim 9 provides that the extremophile is a thermophile. Claim 9 is obvious over the proposed Count in view of at least Brennan (1996) at 31, which suggests preparing libraries to hyperthermophiles, a species of thermophile. |
| **10.** The method of claim 9 wherein the extremeophiles are selected from the group consisting of hyperthermophiles, psychrophiles, halophiles, psychrotrophs, alkalophiles, and acidophiles. | Claim 10 is dependent on claim 9. Claim 10 further recited selected extremeophiles. Claim 10 is obvious over the proposed Count in view of at least Brennan (1996) at 31, which suggests preparing, for example, an expression library to hyperthermophiles. |

| '673 Claim | Count |
|---|---|
| **11.** The method of claim 1 wherein the bioactive substrate comprises C12FDG. | Claim 11 is dependent on claim 1. Claim 11 further provides that the detection substrate is C12-fluorescein-di-β-D-galactopyranoside (C12FDG). This was a commonly used detection substrate. *See, e.g.*, Plovins *et al.*, *Appl. Environ. Microbiol.* 60: 4638-41 (1994). Therefore, claim 11 is obvious over the proposed Count. |
| **12.** The method of claim 1, wherein the bioactive substrate comprises a lipophilic tail. | Claim 12 is dependent on claim 1. Claim 12 further provides that a bioactive substrate, such as C12FDG have a lipophilic tail. Claim 12 is obvious over the proposed Count because the skilled artisan would have known that the attachment of lipophilic carbon chains would impact the solubility of substrates, which would be a useful attribute. See, *e.g.*, Plovins *et al.*, *Appl. Environ. Microbiol.* 60: 4638-41 (1994), which discloses that C12FDG was a useful substrate for β-galactosidase detection by flow cytometry. |

| '673 Claim | Count |
|---|---|
| **13.** The method of claim 1, wherein the samples are heated before step b). | Claim 13 is dependent on claim 1. Claim 13 further provides the step of heating the cells to prevent or reduce extraneous activity. Claim 13 is obvious over the proposed Count because cell fixation methods (*e.g.*, heat and time, permeabilization agents, etc.) was known in the art. *See, e.g.*, R. E. Cunningham, "Flow Cytometry" in MOLECULAR BIOMETHODS HANDBOOK 653-667(Humana Press, 1998) and U.S. Patent No. 5,321,130, at col. 12, ll. 9-35, which describes heating, *inter alia*, can be used to kill cells and allow entry of the fluorescent substrate into the cells. |
| **14.** The method of claim 11, wherein the heating is at about 70°C. | Claim 14 is dependent on claim 11. Claim 14 further provides that the heating should be at 70°C. Claim 14 is obvious over the proposed Count because heating at 70°C is expected by the skilled artisan to kill the cells without denaturing the proteins. *See, e.g.*, U.S. Patent No. 5,321,130 at col. 12, ll. 9-35. |

| '673 Claim | Count |
|---|---|
| **15.** The method of claim 14, wherein the heating occurs at about 30 minutes. | Claim 15 is dependent on claim 14. Claim 15 further provides that the heating step be performed for a period of 30 minutes. Claim 15 would have been obvious over the proposed Count, because typically heat fixation is performed for a period of time depending on the temperature to insure the death of the cell. *See, e.g.,* U.S. Patent No. 4,225,669 at col. 4, ll. 59-66 for staining periods for bacteria and *See, e.g.,* U.S. Patent No. 5,321,130 at col. 12, ll. 9-35. |
| **16.** The method of claim 1, wherein the fluorescent analyzer comprises a FACS apparatus. | Claim 16 is dependent on claim 1. Claim 16 further recites that the fluorescent analyzer is a FACS apparatus. Claim 16 is obvious over the proposed Count, because it was known that FACS was one of the most common high throughput methodologies available for screening cells. *See, e.g.,* Fouchet *et al.*, *Biol. Cell.* 78: 95-109 (1993). |
| **17.** The method of claim 1, wherein the expression library is biopanned before step b). | Claim 17 is dependent on claim 1. Claim 17 further provides that the expression library is obtained by genomic biopanning. Claim 17 is obvious over the proposed Count because tagging nucleic acids with fluorescently labeled oligonucleotide probes ( referred to as "biopanning") was known. *See, e.g.,* Wallner *et al.*, *Cytometry* 14: 136-143. |

| '673 Claim | Count |
|---|---|
| **18.** The method of claim 1, including the additional steps of: subjecting an enzyme encoded by the DNA identified in step d) to directed evolution comprising the steps of:<br>a) subjecting the enzyme to non-directed mutagenesis; and<br>b) screening mutant enzymes produced in step a) for a mutant enzyme. | Claim 18 is dependent on claim 1. Claim 18 further provides for subjecting the DNA encoding the enzyme to random mutagenesis and screening the mutants derived thereby. Claim 18 is obvious over the proposed Count because methods of performing random mutagenesis and methods of screening the mutants were known to the skilled artisan. *See, e.g.*, James D. Watson *et al.*, eds. RECOMBINANT DNA, "In Vitro Mutagenesis," 191-202 (2nd ed., W. H. Freeman & Co., New York, 1992). |
| **19.** The method of claim 1, wherein the prokaryotic expression library is normalized before step b). | Claim 19 is dependent on claim 1. Claim 19 further provides for the DNA library to be normalized in their representation of the genome populations from the original examples. Normalization of DNA libraries was known and described in the art and thus Claim 19 is obvious over the proposed Count. *See, e.g.*, Soares *et al.*, *Proc. Nat'l. Acad. Sci. USA* 91: 9228-32 (1994) and Patanjali *et al.*, *Proc. Nat'l. Acad. Sci. USA* 88: 1943-7 (1991). |
| **20.** The method of claim 1, further comprising co-encapsulating an indicator cell in step b). | Claim 20 is dependent on claim 1. Claim 20 further provides that the cell be encapsulated with a second indicator cell. Numerous methods of encapsulating cells were known at the time. *See, e.g.*, Weaver *et al.*, *Biotechnology* 6: 1084-9 (1988); and U.S. Patent No. 4,801,529 (Abstract). |

| '673 Claim | Count |
|---|---|
| **21.** The method of claim 1, wherein the library is a prokaryotic expression library. | Claim 21 is dependent on claim 1. Claim 21 further provides that the expression library is prepared in a prokaryotic cell system. Claim 21 is obvious over the proposed Count because expression libraries were commonly prepared in *E. coli*, which is an example of a prokaryotic cell. *See, e.g.*, Sambrook *et al.* (1989). |
| **22.** A method for identifying bioactivities or biomolecules using high throughput screening of prokaryotic genomic DNA in a culture-independent system comprising:<br>a) generating a prokaryotic expression library containing a plurality of clones, wherein the DNA for generating the library is obtained from a mixed population of organisms;<br>b) inserting a bioactive fluorescent substrate into the clones of the library, wherein the substrate is fluorescent in the presence of a bioactivity or biomolecule;<br>c) screening the clones with a fluorescent analyzer that detects bioactive fluorescence; and<br>d) identifying clones detected as positive for bioactive fluorescence, wherein fluorescence is indicative of DNA that encodes a bioactivity or biomolecule. | Claim 22 is an independent claim. Claim 22 is a species of the genus of claim 1. Claim 22 further provides for a method of identifying molecules using a culture-independent system. Claim 22 is obvious over the Count in view of at least Brennan (1996) at 31, which suggests that culturing can be bypassed. *See also,* Somerville *et al.*, *Applied & Enviro. Microbiol.* 55: 548-54 (1989). |

| '673 Claim | Count |
|---|---|
| **23.** The method of claim 22, further comprising encapsulation the clone and the bioactive substrate prior to screening. | Claim 23 is dependent on Claim 22. It is assumed that Claim 23 is meant to read "encapsulation of the clone...". Given this assumption, Claim 23 provides that the clone is encapsulated prior to screening. This step is part of step (b) of claim 1, and thus is obvious in view of the proposed Count. *See,* Somerville *et al.*, *Applied & Enviro. Microbiol.* 55: 548-54 (1989). |

## APPENDIX D

**Comparison of the '672 Application Claims with Proposed Count**

| New Claim in '672 Application | Count |
|---|---|
| **46**. A method for identifying polypeptides having a desired activity using high throughput screening of genomic DNA comprising:<br>a) providing an expression library containing a plurality of clones, wherein the DNA for generating the library is obtained from a mixed population of organisms;<br>b) enclosing a fluorescent substrate and at least one clone from the library in a gel microdroplet, wherein the substrate is fluorescent in the presence of the polypeptide having the desired activity;<br>c) screening the microdroplet with a fluorescent analyzer that detects fluorescence; and<br>d) identifying clones detected as positive for fluorescence, wherein fluorescence is indicative of DNA that encodes the polypeptide having the desired activity. | Claim 46 is identical to the second alternative of the Count. |

| New Claim in '672 Application | Count |
|---|---|
| **47.** The method of Claim 46, wherein the polypeptide is an enzyme selected from the group consisting of lipases, esterases, glycosidases, phosphatases, aminotransferases and cellulases. | Claim 47 is dependent on Claim 46. Claim 47 further recites specific enzymes as the polypeptides to be identified by the method. Claim 47 is obvious over the proposed Count given that at least the reference by Brennan suggests these enzymes would be good targets for discovery. *Chem. & Eng. News* 74: 31-33, 31 (1996). |
| **48.** The method of Claim 46, wherein the polypeptide is an enzyme which catalyzes chiral resolutions. | Claim 48 is dependent on Claim 46. Claim 48 further provides for a polypeptide which is an enzyme that is a catalyst for chiral resolutions. Claim 46 is obvious over the proposed Count because Brennan (page 33, col. 3, ll. 9-10) discloses that such enzymes are identified using the method of the Count. |
| **49.** The method of Claim 46, wherein the polypeptide is an enzyme which catalyzes peptide synthesis. | Claim 49 is dependent on claim 46. Claim 49 further recites a polypeptide that is identified by the methods which catalyze peptide synthesis. Claim 49 is obvious over the proposed Count given at least Brennan (1996) at 31, which teaches that aminotransferases, enzymes involved in polypeptide synthesis, are contemplated for isolation. |

| New Claim in '672 Application | Count |
|---|---|
| **50.** The method of Claim 46, wherein the library is generated in a prokaryotic cell. | Claim 50 is dependent on claim 46. Claim 50 further provides that the expression library is prepared in a prokaryotic cell. Claim 50 is obvious over the proposed Count given that expression libraries were commonly prepared in prokaryotic cells, such as *E. coli*. *See, e.g.*, Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL 17.11-17.36 (2nd ed. Cold Spring Harbor 1989). |
| **51**. The method of Claim 50, wherein the prokaryotic cell is gram negative. | Claim 51 is dependent on Claim 50. Claim 51 further provides that the expression library be prepared in a gram negative cell. Claim 51 is obvious over the proposed Count given that expression libraries were commonly prepared in *E. coli*, a gram negative prokaryote. *Id.* *See also*, U.S. Patent No. 5,149,639 at col. 8 *et seq.*: "Example 5 Construction of a Genomic Library of *Streptomyces antibioticus* ATCC 11891 in pNJ1 and introduction into *E. coli*." |
| **52.** The method of Claim 51, wherein the prokaryotic cell is *E. coli*. | Claim 52 is dependent on claim 51. Claim 52 further provides that the expression library be prepared in *E. coli*. Claim 52 is obvious over the proposed Count given that expression libraries were commonly prepared using *E. coli*. *Id.* and *see also* Example 5 of U.S. Patent No. 5,149,639. |

| New Claim in '672 Application | Count |
|---|---|
| **53.** The method of Claim 46, wherein the expression library contains DNA obtained from extremophiles. | Claim 53 is dependent on claim 46. Claim 53 further recites that the expression library DNA is from extremophiles. Claim 53 is obvious over the proposed Count in view of at least Brennan, at 31 (1996), which suggests preparing libraries from extremophiles. |
| **54.** The method of Claim 53, wherein the extremophiles are thermophiles. | Claim 54 is dependent on Claim 53. Claim 54 further recites a species of extremophile, *i.e.*, thermophiles. Claim 54 is obvious over the proposed Count in view of at least Brennan (1996) at 31, which suggests preparing libraries to hyperthermophiles, a species of thermophile. |
| **55**. The method of Claim 54 wherein the extremophiles are selected from the group consisting of hyperthermophiles, psychrophiles, halophiles, psychrotrophs, alkalophiles, and acidopiles. | Claim 55 is dependent on claim 54. Claim 55 further recites species of extremophiles, including for example "hyperthermophiles". Claim 55 is obvious over the proposed Count in view of at least Brennan (1996) at 31, which suggests preparing expression libraries to hyperthermophiles. |
| **56.** The method of Claim 46, wherein the fluorescent analyzer comprises a FACS apparatus. | Claim 56 is dependent on claim 46. Claim 56 further recites that the fluorescent analyzer is a FACS machine. Claim 56 is obvious over the proposed Count, because the skilled artisan would know that FACS was one of the common high through methodologies available for screening cells. *See, e.g.*, Fouchet *et al.*, *Biol. Cell.* 78: 95-109 (1993). |

| New Claim in '672 Application | Count |
|---|---|
| **57**. The method of Claim 46 including the additional steps of: subjecting an enzyme encoded by the DNA identified in step d) to directed evolution comprising the steps of:<br>a) subjecting the enzyme to non-directed mutagenesis; and<br>b) screening mutant enzymes produced in step a) for a mutant enzyme. | Claim 57 is dependent on claim 46. Claim 57 further recites the step of subjecting the DNA encoding the enzyme to non-directed mutagenesis and screening the mutants derived thereby. Methods of performing random mutagenesis and methods of screening the effects were known to the skilled artisan. *See, e.g.*, James D. Watson *et al.*, eds. RECOMBINANT DNA, "In Vitro Mutagenesis," 191-202 (2nd ed., W. H. Freeman & Co., New York 1992). Therefore, claim 57 is obvious over the proposed Count. |
| **58.** The method of claim 46, wherein the DNA for generating the library is genomic DNA from a prokaryote. | Claim 58 is dependent on Claim 57. Claim 58 further recites that the genomic DNA is prokaryotic genomic DNA. Claim 58 is obvious over the proposed Count because the skilled artisan would have known how to obtain genomic DNA from a prokaryote and how to make expression libraries therefrom. *See, e.g.*, Sambrook *et* al., MOLECULAR CLONING: A LABORATORY MANUAL 15.105-15.108 (2nd ed., 1989). *See also*, Example 5 of U.S. Patent No. 5,149,639. |

| New Claim in '672 Application | Count |
|---|---|
| **59.** The method of claim 58, wherein the DNA for generating the library is obtained using a culture-independent system. | Claim 59 is dependent on Claim 58. Claim 59 further recites a method which uses a culture-independent system. Claim 59 is obvious over the proposed count because the skilled artisan would have appreciated the value of performing the method in a culture-independent system in view of at least Brennan (1996) ("Bypassing the culture hurdle. . . .). *See also,* Somerville *et al., Applied & Enviro. Microbiol.* 55: 548-54 (1989). |